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BIOCHEMICAL ANALYSES OF PRESERVED *OCTOPUS GIGANTEUS* TISSUE

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ABSTRACT: Comparative amino acid analyses of *Octopus giganteus* tissue and a variety of control tissues of *Stenella plagiodon, Architeuthis dux, Delphinapterus leucas, Octopus dolfleini,* and *Octopus bimaculoides,* were carried out. The amino acid composition of *O. giganteus* tissue differed significantly from all other specimens, showing a very high proportion of glycine and proline. Comparison with the amino acid composition of known proteins indicates that the *O. giganteus* tissue is mainly collagen and certainly not "whale blubber," as was suggested soon after the discovery of the *O. giganteus* carcass on a Florida beach in late 1896. Comparative determinations of Cu and Fe content of *O. giganteus* tissues and controls were inconclusive, but consistent with a cephalopod identification. These analytical results support the original identification of the tissue and carcass by A. E. Verrill as an exceptionally large cephalopod, probably octopus, not referable to any known species.

INTRODUCTION

On November 30, 1896, a large carcass, half buried in the beach sand of St. Augustine, Florida, was discovered by two young boys while cycling on Anastasia Beach. The find was reported immediately to DeWitt Webb, a local medical doctor with a great interest in natural history. The carcass was washed out to sea and beached twice before Webb was able to obtain a team of horses and move the carcass high enough up the beach to prevent further flotation. Examination of the carcass was made—and photographs were taken—by several people, including Webb, who estimated that the part of the carcass visible above the sand weighed 4,500 kg, and measured 7 meters long by 1.2 meters high by 5.5 meters wide (Fig. 1). Webb identified the carcass as octopus, and, realizing the importance of this discovery, sent descriptive letters to several zoologists, including cephalopod expert A. E. Verrill at Yale University. Verrill promptly published a short note in the *American Journal of Science* (Verrill 1897a), concluding that the carcass was

that of a giant squid, but much larger than the specimens he had examined in Newfoundland in 1870.

Webb forwarded additional data, photographs, and tissue samples to Verrill and to William Healey Dall, curator of molluscs at the U.S. National Museum, in Washington, D.C. On the strength of this additional information, Verrill changed his mind, publishing two articles (Verrill 1897b, 1897c) describing the giant octopus. He calculated that the arms were 20 to 30 meters in length by 0.5 meters in diameter at the base. He named the new animal *Octopus giganteus.*

This identification was greeted with widespread skepticism, and eventually Verrill retracted his original identification (Verrill 1897d, 1897e, 1897f)—pointing out, however, that other zoologists who had examined the tissue still believed that it represented an unknown cephalopod related to the octopus. One of the last pronouncements was by F. A. Lucas, of the U.S. National Museum, who stated: "The substance looks like blubber and smells like blubber, and it is blubber, nothing more nor less." The matter rested there until 1957, when Forrest G. Wood, a marine mammalogist and then curator at Marineland and the Marineland Research Laboratory, Florida, became interested in the matter. He subsequently discovered that the Smithsonian Institution's National Museum of Natural History still possessed a large jar containing the *Octopus giganteus* tissue. Wood contacted a colleague, cell biologist Joseph F. Gennaro, then at the University of Florida, who obtained some tissue samples from the Museum.

Gennaro carried out comparative histological examination of the tissue, concluding that it most resembled contemporary octopus tissue (Wood and Gennaro 1971). While these results were highly suggestive, further biochemical work was required for an unambiguous identification of the tissue.

Method

A variety of tissue samples identified only by number were prepared and submitted to the author for analysis by Joseph F. Gennaro, Department of Biology, New York University. The identity of each sample was unknown to the analyst.

Amino acid analyses were performed by automated ion exchange chromatography, according to the general procedures of Spackman, Stein, and Moore (1958). Five to 50 mg samples were hydrolyzed in 6 N HCl for 24 hours at 110° C. Special precautions recommended for the preparation and hydrolysis of samples were observed (Crestfield, Moore, and Stein 1962, Moore and Stein 1963).

Small aliquots (no less than 25 mg) were analyzed in each case. Analyses were carried out on a Durrum D-500 amino acid analyzer (Dionex Instrument Corporation, Sunnyvale, California).

Cu and Fe determinations were made by standard atomic absorption techniques by P. Rancurel, Laboratoires de Zoologie et Biologie Marines, Centre D'Étude Des Ressources Animales Marines, Université D'aix-Marseille III, under the auspices of Michel Raynal.

FIG. 1.—DeWitt Webb standing by the St. Augustine, Florida, carcass in this 1896 photograph. The ropes were used to move the large mass away from the ocean water. (National Museum of Natural History, Smithsonian Institution.)

Results

The amino acid analyses of *O. giganteus* tissue and controls are shown in Tables 1, 2, and 3. The Cu and Fe concentrations are shown in Table 4.

Discussion

Table 1 shows the amino acid composition of tissue samples from several species, including the arm, mantle, and fin of the giant squid, *Architeuthis*

TABLE 1.—Comparison of amino acid composition of *O. giganteus* with cetacean and cephalopod tissues.

	Stenella plagiodon (spotted dolphin)	*Octopus giganteus*	*Architeuthis dux* (left tentacle III)	*Architeuthis dux* (mantle)	*Architeuthis dux* (fin)	*Delphinapterus leucas* (skin) (white whale or beluga)
Amount weighed out (mg)	5.2	12.4	18.4	49.0	17.0	18.0
Sample	1M	2M	3M	4M	5M	6M
Mole %						
Asp	10.5	5.9	10.5	11.7	10.3	10.5
Thr	4.6	2.5	4.9	5.4	4.8	4.6
Ser	7.1	3.9	6.0	5.8	5.5	8.0
Glu	17.7	8.8	14.2	16.6	12.6	16.7
Pro	4.7	16.8	6.3	4.7	6.5	4.3
Gly	10.6	34.6	19.2	11.5	20.0	14.2
Ala	7.5	13.4	8.6	9.5	8.6	7.6
Val	6.6	2.4	4.8	5.5	5.3	6.4
Met	2.2	0.4	1.9	2.2	1.9	2.1
Ile	5.1	1.2	4.9	5.9	5.4	4.6
Leu	10.7	2.9	7.8	10.0	8.1	10.4
Tyr	1.2	0.0	0.7	0.6	0.5	1.4
Phe	3.1	1.5	2.6	3.0	3.2	3.5
His	0.3	0.0	+	+	0.2	+
Lys	1.6	0.0	1.0	0.9	0.8	0.5
Arg	6.4	5.8	6.6	6.7	6.5	5.2
Hydroxy-lys	0.0	0.0	0.0	0.0	0.0	0.0

+ Trace detected but not integrated—less than 0.1%.

dux, and *Octopus giganteus.* It is immediately apparent that the *O. giganteus* tissue has an amino acid composition unlike any of the other specimens, particularly with respect to high percentages of glycine and proline and lower quantities of glutamic acid.

An important consideration in interpreting these amino acid analyses is the fact that the *O. giganteus* tissue has been in preservative (formaldehyde, and possibly also ethanol) for 90 years. This circumstance might have some effect on the measured quantities of serine, cysteine, threonine, tryptophan, tyrosine, and methionine, by causing degradation, and thus reducing the measured quantities. Since cysteine and tryptophan were not measured in this procedure, they need not concern us. Consequently, any conclusions based on the levels of serine, threonine, tryosine, and methionine would have to be made with caution.

Further, formaldehyde might produce some cross-linking between amino acids, which could also distort the analyses. It is important, therefore, to include analyses of similar tissues exposed to formaldehyde. Table 2 shows comparable amino acid analyses of squid and octopus tissues which have

TABLE 2.—Comparison of amino acid composition of fresh and formaldehyde-preserved cephalopod tissues.

	Contemporary squid (formaldehyde)	*Octopus dolfleini* (formaldehyde)	*Octopus bimaculoides* (formaldehyde)	Contemporary squid (fresh)	Contemporary octopus (fresh)
Amount weighed out (mg)	7.3	12.1	9.6	11.5	13.9
Sample	7G	8M	9M	8MG	9MG
Mole %					
Asp	12.2	13.9	12.0	12.2	13.5
Thr	5.1	5.5	5.2	6.0	5.7
Ser	5.3	1.2	4.2	6.1	2.7
Glu	16.5	11.0	12.4	16.9	16.1
Pro	4.2	5.1	4.7	5.3	5.4
Gly	8.7	22.2	16.7	8.3	11.4
Ala	9.8	11.2	11.4	8.6	10.1
Val	5.5	7.3	6.1	4.3	6.2
Met	2.6	0.0	1.8	2.1	0.5
Ile	6.0	6.5	7.3	5.8	6.2
Leu	10.5	8.9	11.7	9.8	9.8
Tyr	1.3	0.0	0.0	1.5	0.0
Phe	3.4	+	1.4	3.3	0.4
His	1.2	+	0.0	1.5	0.0
Lys	0.8	4.7	2.6	0.4	7.5
Arg	7.1	2.7	2.3	8.0	4.3
Hydroxy-lys	0.0	0.0	0.0	0.0	0.0
Hydroxy-pro	*	*	*	*	*

8M Tentacle tissue from *Octopus dolfleni martini,* 14 kg, 22 meters tentacle tip to tentacle tip. Specimen obtained off Washington State coast near Seattle. Kept in formalin for about 2 years. Courtesy of Roger Closek, Shedd Aquarium.

9M Tentacle tissue from *Octopus bimaculoides* from waters along the California coast. Preserved in formaldehyde at least since 1968, possibly much longer.

\+ Trace detected but not integrated—less than 0.1%.

* Analysis not done.

been preserved in formaldehyde from 2 to 11 years, as well as fresh tissues. No significant effects on the amino acid composition appear to be attributable to formaldehyde storage for these lengths of time. It should be noted that all octopus tissues contain greater quantities of glycine than tissues from squid of comparable size. The giant squid, *A. dux,* does contain larger quantities of glycine, but significantly less than *O. giganteus.* Purified squid collagen free of other proteins does contain higher amounts of glycine typical of collagen, reported by Kimura, Nagaoka, and Kubota (1969), 30.1 mole % glycine and 9.6% proline. These results suggest that the relative collagen content of both squid and octopus are related to their size, the larger the animal the greater the proportion of collagen. Since these animals have no extensive internal or external skeletal support, this interpretation seems reasonable. However, since squids (including *A. dux*), in contrast to octo-

TABLE 3.—Comparison of amino acid composition of *O. giganteus* with collagen, tendon, and bone.

	Bovine collagen	Human collagen	Bovine achilles tendon	Decalcified bone (formaldehyde)	*O. giganteus*	Decalcified bone
Amount weighed out (mg)			15.6	23.2	14.8	19.6
Sample			10MG	11MG	12MG	13MG
Mole %						
Asp	7.5	4.2	7.2	6.6	7.2	6.5
Thr	2.2	1.8	3.4	3.2	3.1	2.7
Ser	3.4	3.6	3.4	4.2	2.0	2.6
Glu	10.8	7.3	9.6	9.0	8.7	11.1
Pro	16.5	12.8	15.6	14.8	16.5	14.3
Gly	29.3	33.0	30.6	31.1	36.0	35.5
Ala	10.0	11.0	14.0	13.5	13.4	12.1
Val	2.7	2.4	3.0	2.9	2.8	2.6
Met	0.9	0.6	0.4	0.7	0.0	0.5
Ile	2.1	1.0	1.6	1.7	1.5	1.6
Leu	3.2	2.4	3.9	3.8	3.6	4.3
Tyr	1.0	0.03	0.0	0.0	0.0	0.0
Phe	2.4	1.2	0.7	1.2	0.6	0.6
His	0.7	0.5	0.0	0.0	0.0	0.0
Lys	5.0	2.7	3.1	2.5	2.3	2.7
Arg	8.6	5.1	3.3	4.7	2.4	2.8
Hydroxy-lys	*	0.6	+	+	+	+
Hydroxy-pro	14.0	9.0	*	*	*	*

+ Present in small amount, but not calculated.
* Analysis not done.

puses, have some rudimentary internal cartilaginous structural plates in addition to the chitinous vestigial pen, somewhat lesser amounts of collagen may be required to maintain their structural integrity than are required by octopuses of comparable size.

Table 3 shows an additional analysis of *O. giganteus* tissue, and analyses of bovine and human collagen, as well as bovine achilles tendon, decalcified bone stored in formaldehyde, and fresh decalcified bone. The amino acid composition of *O. giganteus* tissue is quite similar to collagen, and almost identical to decalcified bone material. The *O. giganteus* tissue is almost pure collagen, which is precisely what one might expect for an aquatic invertebrate, such as a giant octopus, with a mass of 6,000 kg or more.

Copper-containing hemocyanin is the most important respiratory pigment of molluscs with regard to the number of species which possess it. The iron-containing respiratory pigment hemoglobin is also widely—though irregularly—distributed in this group. However, the Cephalopoda, including squids and octopuses, do not possess it. Consequently, Michel Raynal suggested

TABLE 4.—Comparison of Cu and Fe composition of *O. giganteus* with cetacean and cephalopod tissues.

Sample identification	Sample weight (mg)	Cu (ppm)	Fe (ppm)	Cu/Fe
Stenella plagiodon (spotted dolphin) (muscle)	12.8	300	1,600	0.19
Octopus giganteus	29.1	60	200	0.30
Architeuthis dux (mantle)	5.6	240	560	0.43
Delphinapterus leucas (white whale) (skin)	10.5	330	470	0.70

that analyses of the Cu and Fe content of *O. giganteus* tissues might aid in differentiating between cephalopod and mammalian tissue, since cephalopod tissue should contain far less iron. The Cu and Fe content and the Cu and Fe ratios of selected tissues are shown in Table 4. As expected, the relative content of Fe in *A. dux* tissue is much lower than in porpoise tissue, *Stenella plagiodon.* The content of Cu and Fe in the *O. giganteus* tissue is similar to that in *A. dux,* rather than to that in *S. plagiodon.* The sample of whale skin tissue of *Delphinapterus leucas* shows a Cu content similar to that in the porpoise material, but appears to have a much reduced Fe content. The reason for such a low content of Fe in the whale skin is not clear. In any case, these results are consistent with the identification of *O. giganteus* tissue as a cephalopod.

On the basis of Gennaro's histological studies (Wood and Gennaro 1971) and the present amino acid and Cu and Fe analyses, I conclude that, to the extent the preserved *O. giganteus* tissue is representative of the carcass washed ashore at St. Augustine, Florida, in November of 1896, it was essentially a huge mass of collagenous protein. Certainly, the tissue was not blubber. I interpret these results as consistent with, and supportive of, Webb and Verrill's identification of the carcass as that of a gigantic cephalopod, probably an octopus, not referable to any known species. An absolutely definitive identification may possibly be obtained in the future by analyzing and comparing the amino acid sequences of purified collagen samples and/or by radio-immunoassay.

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